

WE CLAIM:

1. A process for the non-fermentative production of KDG or DKG from a carbon source comprising, enzymatically oxidizing the carbon source by at least one oxidative enzymatic activity to yield KDG or DKG .
2. The process of Claim 1 wherein said KDG is further converted to erythorbate.
3. The process of Claim 1 comprising oxidizing the carbon source by a first oxidative enzymatic activity to yield a first oxidative product and oxidizing said first oxidative product by a second oxidative enzymatic activity to yield KDG.
4. The process of Claim 3 wherein said first oxidative enzymatic activity is a GDH activity and said second oxidative enzymatic activity is an GADH activity.
5. The process of Claim 1 that proceeds in an environment comprising host cells.
6. The process of Claim 5 wherein said host cell is non-viable.
7. The process of Claim 5 wherein said host cell is viable.
8. The process of Claim 5 wherein at least one oxidative enzyme is bound to said host cell membranes.
9. The process of Claim 1 wherein at least one oxidative enzymatic activity is in solution.
10. The process of Claim 8 wherein said host cell comprises a mutation in the naturally occurring nucleic acid encoding a KDGDH activity.
11. The process of Claim 5 wherein said host cell is an member of the family Enterobacteriaceae.

12. The process of Claim 11 wherein said member is a Pantoea species.

13. The process of Claim 1 wherein at least one oxidative enzymatic activity
5 immobilized.

14. The process of Claim 3, further comprising the steps of enzymatically oxidizing
the KDG by at least one oxidative enzyme to an oxidation product; and enzymatically
reducing said oxidation product by at least one reducing enzyme to 2-KLG.

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15. A process for the non-fermentative production of 2-KLG from a carbon source,
comprising the following steps in any order, enzymatically oxidizing the carbon
source by at least one oxidative enzymatic activity to an oxidation product; and
enzymatically reducing said oxidation product by at least one reducing enzymatic
15 activity to 2-KLG.

16. The process of Claim 15 wherein said carbon source is KDG.

17. The process of Claim 15 wherein said oxidative enzymatic activity requires an
20 oxidized form of an enzymatic co-factor and said reducing enzymatic activity requires
a reduced form of said enzymatic co-factor and wherein said oxidized form of said
co-factor and said reduced form of said co-factor are recycled between at least one
oxidizing step and at least one reducing step.

25 18. The process of Claim 15 comprising the following steps in any order:

- a. enzymatically oxidizing the carbon source by a first oxidative enzymatic
activity to a first oxidation product;
- b. enzymatically oxidizing the first oxidation product by a second oxidative
enzymatic activity to a second oxidation product;
- c. enzymatically oxidizing the second oxidation product by a third oxidative
enzymatic activity to a third oxidation product; and

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- d. enzymatically reducing the third oxidation product by a reducing enzymatic activity to 2-KLG.

19. The process of Claim 18 wherein at least one of said first, second and third
5 oxidative enzymatic activities requires an oxidized form of an enzymatic co-factor and said reducing enzymatic activity requires a reduced form of said enzymatic co-factor and wherein said oxidized form of said co-factor and said reduced form of said co-factor are recycled between at least one oxidizing step and the reducing step.

Sub C3

20. The process of Claim 19 wherein said first oxidative enzymatic activity requires an oxidized form of said enzymatic co-factor.

21. The process of Claim 18 wherein said carbon source is glucose and said first enzymatic activity is a glucose dehydrogenase activity.

22. The process of Claim 21 wherein said glucose dehydrogenase activity is obtainable from a bacterial, yeast or fungal source.

23. The process of Claim 22 wherein said glucose dehydrogenase activity is
20 obtainable from a source including *T. acidophilum*, *Cryptococcus uniguttalatus* and *Bacillus* species.

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24. The process of Claim 19 wherein each of said first, said second enzyme and said third enzyme is a dehydrogenase activity.

Sub C4

25. The process of Claim 19 wherein at least one of said first, said second, said third and said fourth enzymatic activities are immobilized.

26. The process of Claim 19 wherein at least one of said first, said second, said
30 third and said fourth enzymatic activities are in solution.

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27. The process of Claim 25 wherein said second enzyme is a GADH activity.

28. The process of Claim 25 wherein said third enzyme is KDGDH activity.

29. The process of Claim 25 wherein said fourth enzyme is a reductase activity.

5 30. The process of Claim 29 wherein said reductase activity is obtainable from a bacterial, yeast or fungal source.

31. The reductase activity of Claim 29 wherein said source includes *Corynebacterium* and *Erwinia*.

32. The process of Claim 31 wherein said reductase activity is 2,5 DKG reductase.

33. The process of Claim 18 wherein said first oxidation product is gluconate, said second oxidation product is 2-KDG, and said third oxidation product is 2,5-DKG.

34. The process of Claim 18 that proceeds in an environment comprising recombinant host cells.

35. The process of Claim 34 wherein said host cell is viable.

36. The process of Claim 34 wherein said host cell is non-viable

37. The process of Claim 34 wherein said recombinant host cells comprise members of Enterobacteriaceae.

38. The process of Claim 34 that proceeds in an environment comprising recombinant host cell membranes and wherein at least one of said first, said second and said third enzymes are bound to said host cell membranes.

39. The process of Claim 37 wherein said recombinant host cell is a *Pantoea* species.

40. The process of Claim 39 wherein said recombinant host cell is *Pantoea citrea*.

41. The process of Claim 40 wherein said recombinant host cell has a mutation of at least one naturally occurring dehydrogenase activity.

42. The process of Claim 41 wherein said mutation is in a membrane bound GDH activity.

43. The process of Claim 41 wherein said host cell further comprises nucleic acid encoding a heterologous GDH activity.

44. The process of Claim 43 wherein said heterologous GDH activity is obtainable from *T. acidophilum*, *Cryptococcus uniguttalatus*, or a *Bacillus* species.

45. The process of Claim 18 wherein said oxidized form of said enzymatic cofactor is NADP+ and said reduced form of said enzymatic cofactor is NADPH.

46. The process of Claim 18 wherein said oxidized form of said enzymatic cofactor is NAD and said reduced form is NADH.

47. The process of Claim 1, Claim 15 or Claim 18 that is continuous.

48. The process of Claim 1, Claim 15 or Claim 18 that is batch.

49. The process of Claim 1, Claim 15 or Claim 18 that proceeds in an environment comprising organic solvents.

50. The process of Claim 1, Claim 15 or Claim 18 that proceeds in an environment comprising long polymers.

51. The process of Claim 14, Claim 15 or Claim 18 further comprising the step of obtaining ASA from said 2-KLG.

6PH

52. A host cell comprising nucleic acid having a mutation in the gene encoding GHD activity.

53. A host cell comprising nucleic acid having a mutation in the gene encoding
5 KDGDH activity.

54. The host cell of Claims 52 or 53 that is a Pantoea species.

55. The host cell of Claim 52 further comprising nucleic acid encoding a
10 heterologous GDH activity.

56. The host cell of Claim 55 further comprising nucleic acid encoding a
heterologous reductase activity.

15 57. The process of Claim 1 optionally comprising the step of recovering said KDG or
DKG.

58. The process of Claim 14, Claim 15 or Claim 18 wherein said 2-KLG is further
purified via electrodialysis.

59. The process of Claim 45 or Claim 46 wherein said co-factor is purified via
nanofiltration.

60. The process of Claim 18 that proceeds in an environment comprising salt.

61. The process of Claim 60 wherein the salt includes ammonium sulfate, sodium
acetate, ammonium acetate, ammonium chloride, sodium sulfate, potassium
phosphate, sodium phosphate, sodium chloride, , KCl, NH₄Cl, K₂SO₄ and NaI.

62. The process of Claim 60 that comprises a salt concentration between 0 mM and
500mM.

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